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# Protective Effects of *Melissa officinalis* (Lemon Balm) on Sperm Parameters and Spermatogenesis Quality in Rats Exposed to Lead

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## Abstract

**Background:** Some medicinal plants with their antioxidant properties have protective effects on sperm parameters and neutralize the harmful effects of lead exposure.
**Objectives:** The present study aimed to determine the effects of Melissa on sperm parameters and spermatogenesis quality in adult rats exposed to lead.
**Methods:** This animal experimental study was conducted on 45 male Wistar rats weighing 180 ± 20 g, which were allocated randomly into five groups (n=9): distilled water gavages (control) (0.5 ml); intraperitoneal distilled water (i.p. control) (0.5 ml); Melissa (100 mg/kg/bw); lead acetate (10 mg/kg/bw); and lead acetate (10 mg/kg/bw) plus Melissa (100 mg/kg/bw). After a 30-day treatment, the animals were sacrificed. Sperm parameters (count, motility, morphology, and chromatins assay) and weighing of the epididymis and testis were performed.
**Results:** The results showed that in the lead acetate plus Melissa group, epididymis weight (0.37gr vs. 0.32gr), testis weight (1.40 vs. 1.28g ), sperm motility (4.44% vs. 33.2 %(, and viable sperm (90 vs. 50) were higher than the lead group. However, testicular weight, normal morphology, and DNA percentage showed insignificant differences among the study groups (P > 0.05).
**Conclusions:** The results of the present study indicate the protective effects of *Melissa officinalis* on some sperm parameters and spermatogenesis quality in rats exposed to lead.

*Keywords:* Antioxidants, Lead Acetate, Melissa, Spermatogenesis

## 1. Background

Male infertility is one of the most important dilemmas in human life, and abnormalities in sperm quantity and quality can be seen in 40% - 50% of infertility cases (1). Several factors, including lead exposure, contribute to these problems (2). Lead has been used in building paint, batteries, electronics industries, ceramics, jewelry, prints, ammunition, and plastic PVC in two organic and inorganic forms. The inorganic form of lead can be absorbed via respiratory or digestive systems and can affect nervous, circulatory, urinary, digestive, and reproductive systems. Generally, lead eliminates slowly and its biologic half-life in soft tissue is about 24 - 40 days (3-6).

Long-term exposure to lead can decrease male fertility rate, sperm count (2), sperm adhesion ability (2), testis weight, somniferous diameter (2), and sperm viability (7), which are chronologically reversible (2). Oxidative stress is a common pathology seen in approximately half of all infertile men (8). These effects may be related to activation of reactive oxygen species (ROS) production (9). Oxidative stress is an imbalance between free-radical production, especially ROS and antioxidant systems (10). ROS, defined as including oxygen ions, free radicals, and peroxides are generated by sperm and seminal leukocytes (11). Human spermatozoa are susceptible to peroxidative damage because of their high levels of unsaturated fatty acids with multiple double-bond and their ability to generate ROS, mainly superoxide anion and hydrogen peroxide. On the other hand, antioxidants, such as vitamins E and C and carotenoids, can preserve appropriate pro-oxidant-antioxidant balance (oxidative stress) and prevent the DNA damage by preserving sperm's cellular oxidative stress (9).

Medicinal plants are the main sources of natural antioxidants (12). Some medicinal plants, such as onion (13) and *Fumaria parvifloral* (14), have protective effects on sperm parameters (15).

*Melissa officinalis* belongs to the family of Lamiaceae, a large group of medicinal plants. *M. officinalis* is a perennial plant that grows all over the Mediterranean region. The leaves of *M. officinalis* have been used in folk medicine, especially in Turkey and Iran, for the treatment of some disease (12).

Phytochemical study demonstrates the existence of different components in *M. officinalis* such as poly acids (rosmarinic acid, caffeic acid, and protocatechoic acid), aldehides monotherpenoid oil, terepens, flavonoids, and tanan (16). Antioxidant properties of this herb are related to components such as flavonoids, carnosic, and tritrinic fatty acids. On the other hand, the hydroxinamic acid drivates, komarim acid, glocoside eriodictyol7-0, and luteolin acid salvianic are related to the antioxidant capacity of this herb (15).

Regarding the limited studies investigating the effects of *M. officinalis* on sperm parameters in lead-exposed male rats and the plant's antioxidant properties.

## 2. Objectives

The present study was conducted to determine the protective effect of *M. officinalis* on spermatogenesis and sperm parameters.

## 3. Methods

This animal experimental study was conducted to investigate the protective effects of *M. officinalis* on sperm parameters and spermatogenesis quality in rats exposed to lead. The study protocol was approved at the Applied Pharmaceutical Research Center of Tabriz University of Medical Sciences in 2014 (with ethic code number 741 in August 2013), and researchers considered the national law on the care and use of laboratory animals.

According to the Declaration of Helsinki in animal study (7), attention was paid to the environmental protection and welfare of laboratory animals.

Accordingly, in this study, we adhered to the following:

1. Minimum sample to run and correct conclusion was used.

2. During the study, as well as before and after the study, tried to create the right conditions for feeding and life samples.

3. All phases of the work and living conditions were conducted under the supervision of a veterinarian consultant.

### 3.1. Sample Collections

In the present study, the sample size was determined according to a previous study by Golshan Iranpour et al. (17). Moreover, the sample size was estimated by STATA software: $\alpha$ = 0/05, $\beta$ = 80%, $Sd_1$ = 9, $Sd_2$ = 1, $M_1$= 67, $M_2$ = 56 were considered without design effect, 30 (five rats in each group), and with design effect, 45 (nine rats in each groups).

A total of 45 male Wistar rats, 8 - 10 weeks old and ranging in weight from 180 $\pm$ 20 g, were purchased from Tehran Pasteur Institute. The animals were treated in the animal house of the Pharmaceutical Research Center at Tabriz University of Medical Sciences for one week under 12 hours of light and 12 hours of darkness at 25°C and humidity of 40% - 70% based on the principles of Laboratory Animal Care. The rats were weighed, numbered, and then allocated randomly into 5 groups of 9 rats each with similar average weights using the SPSS software (SPSS Inc., Chicago, IL, USA).

### 3.2. Herbal Extraction Method

Accordingly, the Melissa herb was collected in spring from Tabriz (Sahand hillside) and confirmed by two herbalists from the faculty of pharmacy of the Tabriz University of Medical Sciences, then dried in the shade after washing and grounded (40 mesh). In this stage, 1500 g of the grounded leaf powder was mixed with 2500 ml of ethyl alcohol (70%) and was kept in a dark place for 48 hours. The contents of the Erlenmeyer flasks were stirred for 25 minutes once every 4 hours. After 48 hours, the contents of the Erlenmeyer flasks were smoothed by filter paper. Then, the smoothed fluid was extracted in the rotary evaporation apparatus under vacuum and 36 °C temperature. The concentrated extract was poured into a sterile Petri dish and dried in an oven at 36°C. Dried powders were collected and prepared at a concentration of 100 mg using sterile distilled water.

### 3.3. Intervention Protocol

Interventions were carried out 3 days a week for one month for the following groups:

1. Control group (C1): 0.5 mL of distilled water by gavage

2. Intraperitoneal (i.p.) control group (C2): 0.5 mL of distilled water intraperitoneally

3. Lead acetate (L):10 mg/kg/body weight (bw) intraperitoneally

4. *M. officinalis* (M):100 mg/kg/bw by gavage

5. Lead acetate + M. *officinalis* (L + M): as above dose and route

Twenty-four hours after the end of intervention, all the animals were weighed and, with consideration of ethic criteria, anesthetized with ether and sacrificed. Their right reproductive organs consisting of epididymis and testis were removed and weighed by a digital scale with 0.001 accuracy (Mettler-Toledo B303-S Co, Swiss) and dissolved in phosphate-buffered saline (PBS). The digital scale was calibrated before each measurement. Subsequently, sperm samples were collected from the distal part of the

epididymis and examined based on sperm count, motility, and morphology (18). The sperm were released in the medium provided by GibcoHam's F10 Company. The medium osmolarity and pH were set at 285 mmol and 7.2 - 7.4, respectively. Sperm motility was examined by placing a drop of the medium on the slide using an optical microscope with a magnification of × 40.

Another smear was fixed on the slides by Carnoy's solution and stained with acridine orange for the detection of sperm with normal DNA based on Tejada (19).

The percentage of sperm with normal (green color) and denaturated DNA (red color) was reported (20).

A drop of medium containing sperm was placed on a 20 × 20 mm glass slide, and then a cover slip was placed on top. To assess the sperm condition, the prepared slides were observed under an optical microscope (Olympus bx40, Japan) with the object lens of × 40, and the number of motile and nonmotile sperm were counted (200 spermatozoa per slide) and their ratio (sperm motility) was determined.

Regarding the vital sperm membrane permeability, the number of live and dead sperm were counted. For eosin–nigrosin staining, a drop of sperm-containing medium was placed on the glass slides and fixed with alcohol. Eosin cannot penetrate the membrane of living cells. Therefore, viable cells remained uncolored, and the dead sperm were seen in pink color. At least 300 spermatozoa were evaluated per slide, and the percentage of live sperm cells was calculated (18).

In order to increase the accuracy of observation, one observer performed different laboratory tests. All stages of the experiment were blind, and after the intervention, the laboratory expert was unaware about the study groups.

### 3.4. Statistical Analysis

To determine normality of data, descriptive indices (skewness, kurtosis) were used. All the data in the study have been reported as IQR ± median and frequency. The normality assumptions of data were checked using one sample kurtosis and skewness tests (K - S) and for normal and non-normal data parametric and nonparametric tests were used, respectively. The data were analyzed by Kruskal - Wallis, Wilcoxon, Mann - Whitney U test, and chi-square tests using SPSS21 Inc., Chicago software. The P-value < 0.05 is considered as significant difference in this study.

## 4. Results

Regarding K-S, the distribution of data was not normal. Therefore, nonparametric tests were used to analyze the variables.

### 4.1. Weights of Body and Reproductive Organs

The average weight of rats were matched in the beginning of the study (P = 0.51), but the Kruskal-Wallis test showed a significant difference in weight gain among study groups (P = 0.003). According to the Mann-Whitney U test, these differences were significant among C1 with L and C1 with L + M (P = 0.02 and P = 0.006, respectively); moreover, the highest weight gain was observed in the C1 and M groups, respectively, whereas the lead acetate group showed the lowest weight gain. In comparison, the Wilcoxson test highlighted a significant difference in weight gain for the C2 and M groups (P = 0.007 and P = 0.02, respectively) (Table 1).

The results of the study showed an insignificant difference among the study groups based on testis weight (P = 0.87). However testis weight in the L + M group was higher than the L group (1.40 vs. 1.28 g). Moreover, findings of the study indicated that epididymic weight was significantly different among the study groups (P = 0.006), in which the C1 and M groups had the highest and lead acetate the lowest weight. In comparison, using the Mann-Whitney U test, a significant difference was observed between the C2 with L and C2 with L + M groups (P = 0.017 and P = 0.005, respectively) (Table 2).

### 4.2. Spermatography

Results showed a significant difference among study groups in terms of sperm motility (P < 0.001), and the highest sperm motility (equal and more than 50%) was seen in C1 and C2 groups. In comparing the groups, C2 had a statistically significant difference with the L + M, L, and M groups (P = 0.009, P = 0.008, and P = 0.008, respectively). Moreover, sperm motility in the L + M group was insignificantly higher than the lead group (4.44% vs. 33.2%) (Table 3).

According to the findings of the study, no significant differences were found among the study groups by the means of percentage of normal morphology (P = 0.21) and normal DNA (P = 0.09) (Table 4), and all observed sperm morphology in the M group was normal (Figure 5).

The percentage of inviable sperm was significantly different among the study groups (P = 0.003). The analysis of data suggested that the maximum number of dead sperm was found in the L group. Moreover, the C1 group had a significant difference with the C2 and L + M groups (P = 0.004). Although viable sperm in the M + L group was more than the lead group (90 vs. 50), this difference was insignificant (Table 4).

There were no obvious side effects in the present study. However, we did not check for any cytotoxic effects of Melissa.

**Table 1.** The Frequency of Weight Gain Among the Study Groups[a,b]

| Group | Weight Before Intervention (gr) | Weight After Intervention (gr) | Weight Gain (gr) | $P_2$ |
|---|---|---|---|---|
| | Median (Percentile 25,75) | Median (Percentile 25,75) | Median (Percentile 25,75) | |
| **Control group (IP)** | 215.00 (162.00,265.00) | 237.50 (195.00,262.75 ) | 31.00 (-4.75,45.00) | 0.05 |
| **Control group (gavage)** | 170.50 (146.25,247.25) | 213.00 (196.25,312.25) | 42.00 (51.50,37.00) | 0.007 |
| **Lead** | 210.00 (167.50,245.00) | 227.00 (171.50,278.50) | 14.22 (-6.50,29.50) | 0.06 |
| **Melissa + lead** | 205.00 (193.50,283.00) | 215.00 (205.00,261.50) | 6.00 (-7.50,33.50) | 1.00 |
| **Melissa** | 210 .00 (190.00,246.50) | 240.00 ( 250.50,261.50) | 17.00 ( 4.00,24.00) | 0.02 |
| **$P_1$** | 0.51 | 0.99 | 0.03 | |

[a] Based on the Kruskal-Wallis test ($P_1 = 0.003$), there was a significant difference among the study groups.
[b] P1: P-value between groups/ P2: P-value in whit groups.

**Table 2.** The Frequency of Testis and Epididymic Weights Among the Study Groups[a]

| Group | Testis Weight, Median (Percentile 25,75) | Epididymic Weight, Median (Percentile 25,75) |
|---|---|---|
| **Control group (IP)** | 1.22 (1.17,1.41) | 0.53 (0.45,0.59) |
| **Control group (gavage)** | 1.37 (1.21,1.48) | 0.41 (0.23,0.62) |
| **Lead** | 1.28 (1.13,1.49) | 0.32 (0.47,0.21) |
| **Melissa + lead** | 1.40 (1.03,1.59) | 0.37 (0.30,0.47) |
| **Melissa** | 1.37 (1.25,1.55) | 0.45 (0.41,0.53) |
| **P** | 0.87 | 0.006 |

[a] $P < 0.05$ was considered as significant based on the Kruskal-Wallis test.

**Table 3.** Spermatography Within the Study Groups[a]

| | Control Group (IP) | Control Group (Gavage) | Lead | *Melissa officinalis* +Lead | *Melissa officinalis* | (P) |
|---|---|---|---|---|---|---|
| **Sperm motility ($\geq$ 50%)** | 9 (100) | 7 (77.6) | 3 (33.2) | 4 (4.44) | 4 (4.44) | 0.001 |

[a] Values are presented as No. (%).

**Table 4.** Frequency of Sperm Viability, Normal Morphology, and Normal DNA Among the Study Groups[a]

| Group | Percentages of inviable sperm | Percentages of normal morphology | Percentages of normal DNA |
|---|---|---|---|
| **Control group (IP)** | 10.00 (16.00, 10.00) | 100.00 (99.00, 100.00) | 12.00 (10.00,19.00) |
| **Control group (gavage)** | 10.00 (5.50,10.00) | 100.00 (99.00, 100.00) | 48.00 (3.50,50.00) |
| **Lead** | 50.00 (12.50,93.50) | 99.00 (98.50,100.00) | 25.00 (13.00, 45.00) |
| **Melissa + lead** | 10.00 (5.00,10.00) | 99.00 (99.00, 100.00) | 10.50 (1.50, 20.50) |
| **Melissa** | 10.00 (4.00,17.50) | 100.00 (100.00, 100.00) | 25.00 (2.75, 54.00) |
| **(P)** | 0.003 | 0.09 | 0.21 |

[a] $P < 0.05$ was considered significant between the study groups based on the Kruskal-Wallis test.

## 5. Discussion

The present study assessed the protective effects of Melissa hydro-alcoholic extract on sperm parameters and quality of spermatogenesis. In the present study, the lead acetate group showed the lowest weight gain in comparison with other groups. Treatment by lead could reduce the growth rate due to several factors, including imbalance

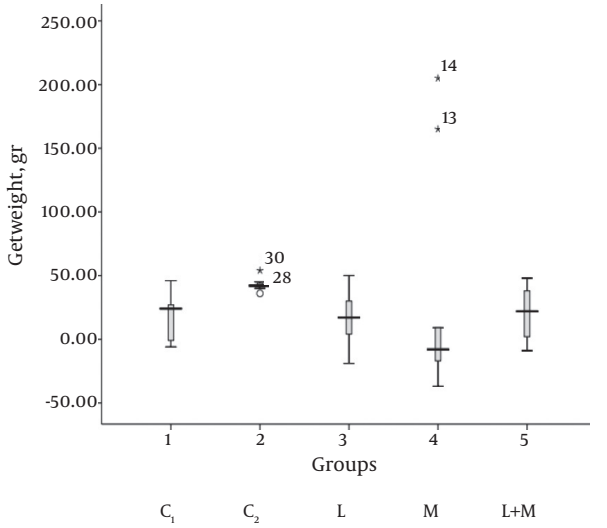



**Figure 1.** The Plot of Weight Gain Among the Study Groups

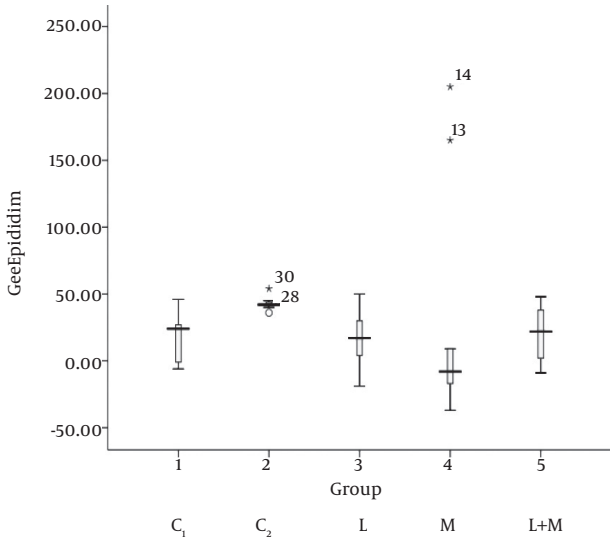



**Figure 2.** The Plot of Epididymic Weights Among the Study Groups

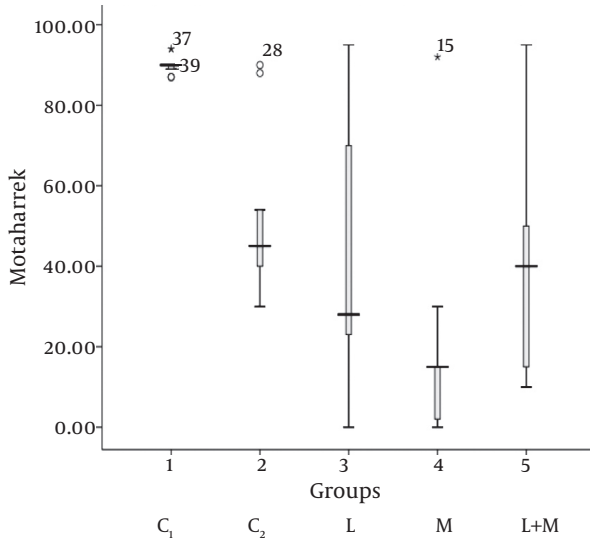



**Figure 3.** The Plot of Spermatography Within the Study Groups

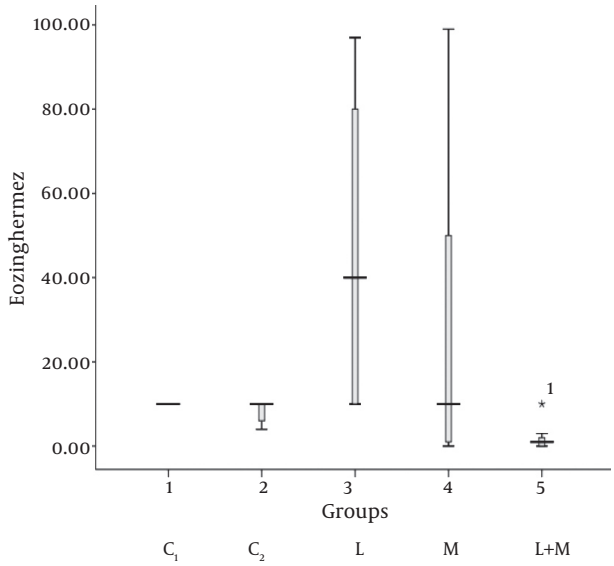
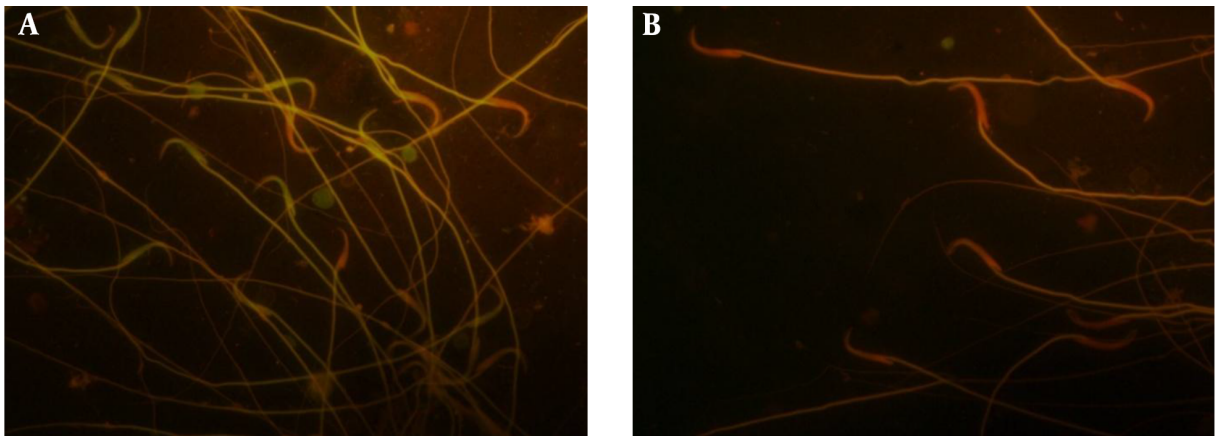



**Figure 4.** The plot of Sperm Viability Within the Study Groups

in the metabolism cycle via change in the zinc element, of which the related enzymes are essential to the metabolism cycle (21).

It was evident that the lead acetate group had the lowest epididymis weight, sperm motility, and inviable sperm rate. Several studies have found that sperm parameters are affected by lead exposure. Mishra and colleagues (22), in their study, demonstrated that administration of 10 mg/kg/mouse of lead acetate for 5 weeks caused an increase in abnormal sperm. Furthermore, in studies by Liva and colleagues (16) and Dorostghoal and colleagues (13), exposure to lead acetate caused a decrease in sperm count. In a study by Priya et al. (23), treatment with lead caused a significant decrease in weight of the testes, epididymis, sperm count, sperm motility, and sperm viability. The lowest weight of reproductive organs in the lead group in the present study may be related to a decrease in germ cells

**Figure 5.** Sperm Integrity




A, Melissa group (100 mg/kg/mouse): Normal DNA marked by green color; B, Lead acetate group (10 mg/kg/mouse): Redness of sperm's head indicates DNA denaturation; Magnified by a multiple of 1000 with acridine orange staining.

and spermatid cells (24). In the present study, the most inviable sperm were seen in the lead group. Al-Attar et al. (25), in their study, demonstrated that high doses of lead exposure caused decreased sperm motility, which confirms the findings of the present study.

Several contributing factors could affect spermatozoid viability, sperm motility, and fertility rate (26-29). Human spermatozoa has a specific sensitivity to peroxidative damage regarding its high levels of double-bond unsaturated fatty acids and its ability to produce ROS, especially superoxide and hydrogen peroxide anions, which activate one of the ROS pathways caused by environmental or physiological factors (9).

Oxidative stress and excess ROS are some of the major causes of DNA fragmentation and damage in spermatozoa (30, 31). Moreover, antioxidants secreted by the reproductive tract protect spermatozoa against the toxic effects of ROS after ejaculation (32). In the present study, the lead acetate group had a significant difference from the lead + Melissa group in terms of sperm viability, and treatment with Melissa caused a remarkable decrease in the percentage of inviable sperm. In addition, a significant difference was seen in sperm motility among the lead acetate group and the L + M and M groups. The elimination or decrease in oxidative damage is probably a mechanism of the Melissa extract on sperm viability and motility. The antioxidant properties of Melissa extract is associated with rosmarinic acid and benzodioxol, which carry out their function by elimination of free radicals (33, 34). Rosmarinic acid belongs to the group of polyphenols, which are strong antioxidants (35) that inhibit lipid peroxidation and, by this mechanism, increase sperm motility (36).

Results of this study showed that sperm motility was better in control groups than the Melissa group. This condition may be related to the amounts of antioxidant in the present dosage of Melissa (100 mg/kg/bw). Puerta Suarez et al. (37) found that *M. officinalis* extract decreased sperm motility and viability in comparison to their control group.

The difference between the Melissa + lead group and control groups in some sperm parameters in the present study may be related to duplication of the stress effect related to i.p. injection and gavage simultaneously in this group. The rats in this group received lead acetate in i.p. form and Melissa extract via gavage. Retana-Marquez et al. (38) demonstrated that different stressors could change masculine sexual behavior in response to acute and chronic stress in male rats. Collodel (39) confirmed the effects induced by stress on meiotic and structural alterations in sperm cells.

### 5.1. Conclusion

As lead has several adverse effects on body organs, researchers propose different strategies, such as attention to food and nutrition, especially antioxidants, to decrease or eliminate these effects.

Regarding the results of the present study, consumption of *M. officinalis* with its antioxidant properties has a positive effect on weight gain, epidydimal weight, percentage of viable sperm, and toxic effects of lead on dead sperm, but in the studied dose, has no effect on other fertility indices.

### 5.2. Limitation

The strength points of the present study were the evaluation of Melissa extract with antioxidant properties on sperm parameters due to lead acetate toxicity in several groups; however, regarding financial limitations, the intervention period, varieties of extracts, and dosage were restricted.

## Footnotes

**Authors' Contribution:** Mehran Mesgari Abbasi: main investigator, design, and carrying out the A-Z of the research; Mahnaz Shahnazi: design and drafting; Mohammad Asgari Jaafarabadi: statistical analysis; Narjeskhaton Dadkhah: design, data collection, interpretation, drafting. Bita Aabdollahi: data collection.

**Conflict of Interest:** None of the authors have any conflict of interest to declare.

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